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Nutrient and Cardenolide Composition of Unextracted and Solvent-Extracted *Calotropis procera*

M. D. Erdman

Solvent extraction of *Calotropis procera* results in a hydrocarbon fraction potentially valuable as a fuel or chemical feedstock. The data presented indicate that residues extracted with solvents have a value as a fibrous animal feedstuff. The nutrient and cardenolide composition of unextracted, hexane-extracted, and hexane and methanol extracted plant and individual plant component residues from *C. procera* were determined. All residues were compared with respect to proximate analysis, amino acid profile, in vitro digestibility, and mineral and cardenolide composition. Hexane extraction of the plant residues generally enhanced the nutrient composition but did not greatly reduce cardenolide levels. Hexane and methanol extracted residues, however, were somewhat lower in nutrient content but essentially cardenolide free. The presumptive nutrient value of extracted residue from *C. procera* supports cultivation of this plant species as a potential fuel and feed resource.

Calotropis procera (Art.) R. Br. (Asclepeadaceae) is a large broadleaf evergreen which grows abundantly in arid and semiarid regions of the world without irrigation, fertilizer, pesticides, or other agronomic practices. This plant is very resistant to fire and coppices profusely (Little et al., 1974; Mahmound et al., 1979a; Saxena and Singh, 1976; Karschon, 1970). Although widely distributed, little commercial use for this plant species has been reported (Vasudevan et al., 1981).

The use of plants as renewable hydrocarbon sources or chemical feedstocks has been reported (Buchanan et al., 1978a,b; Nemethy et al., 1979; Calvin, 1980, 1982). Recently harvested *C. procera* biomass was solvent extracted with hexane and methanol and also demonstrated to be a rich source of hydrocarbons (Erdman and Erdman, 1981) that was comparable to extractables from *Euphorbia lathris* (Nemethy et al., 1979; Calvin, 1980). Although hydrocarbon extraction from *C. procera* has been demonstrated, secondary uses for the extracted residues have not been reported.

The principal toxic components in *C. procera* are cardenolides (Watt and Breyer-Brandwijk, 1965; Brüschweiler et al., 1969a,b; Biedner et al., 1977; Garg, 1979; Nelson et al., 1981; Seiber et al., 1982). Cardenolides are 5βH,14β-hydroxy α,β-unsaturated γ-lactones and are widely distributed in the plant kingdom as glycosides. In small doses they tend to slow and strengthen the heart beat, but excessive doses can cause the heart to stop (Standen et al., 1969). Any proposed cultivation or processing scheme to utilize *C. procera* must consider these highly toxic compounds.

Limited feeding trials of unextracted residues or latex from *C. procera* were shown to cause death in sheep fed 5–10 gm/kg of body weight (Mahmoud et al., 1979a,b), while cattle were unaffected (Canella et al., 1966). Oral administration of *C. procera* flower extract was shown to cause widespread testicular necrosis, reduced levels of protein, RNA, and sialic acid in tissues, and liver damage in the gerbil *Meriones hurrianae* (Garg, 1979). Accidental contact of plant latex with the human eye caused a temporary acute inflammatory reaction and intense photophobia, which subsided with medical treatment (Biedner et al., 1977). The objectives of this work were to determine the nutrient and cardenolide content of unextracted and solvent-extracted *C. procera* residues.

MATERIALS AND METHODS

C. procera plant (aerial portion, ≤2 m in height) and individual plant components were collected in the southern coastal region of Puerto Rico. The components were oven dried (75 °C), Wiley milled, and Soxhlet extracted with

U.S. Department of Agriculture, Agricultural Research Service, Southern Agricultural Energy Center, Tifton, Georgia 31793.

Table I. Proximate Analysis of Unextracted and Extracted *C. procera*

component	crude protein, %	ether extract, %	ash, %	crude fiber, %	nitrogen-free extract, %
Unextracted					
whole plant	15.84[b]	3.28[b]	13.29[b]	18.72[c]	48.87[b]
stems	6.66[d]	2.56[d]	7.23[d]	41.91[a]	41.65[d]
leaves	17.69[a]	3.08[c]	14.56[a]	13.65[d]	51.04[a]
pods	14.47[c]	8.68[a]	8.24[c]	23.69[b]	44.93[c]
Hexane Extracted					
whole plant	13.41[c]	0.43[a]	11.37[b]	24.08[c]	50.72[b]
stems	6.69[d]	0.28[a]	7.66[d]	38.78[a]	46.60[c]
leaves	18.22[a]	0.31[a]	15.07[a]	13.49[d]	52.91[a]
pods	15.19[b]	0.32[a]	8.96[c]	27.27[b]	48.28[c]
Hexane and Methanol Extracted					
whole plant	12.72[c]	0.16[a]	10.27[b]	32.29[b]	44.57[c]
stems	5.31[d]	0.22[a]	6.70[d]	46.55[a]	41.23[d]
leaves	18.88[a]	0.18[a]	15.02[a]	16.10[d]	49.84[a]
pods	14.06[b]	0.18[a]	8.34[c]	29.74[c]	47.68[b]
LSD (0.05)[a]	0.24	0.12	0.52	0.91	1.21
alfalfa[b]	24.8	2.2	10.3	15.6	47.1
euphorbia[b]	14.4	4.1	11.0	13.0	57.5

[a] LSD is defined as the least significant difference between similar components for different treatments. [b] International feed reference no. 100046 and 201884, respectively (National Academy of Sciences, 1979a).

hexane or separately with hexane, followed by methanol as previously reported (Erdman and Erdman, 1981). Proximate analysis of unextracted and extracted residues were performed by standard methods (Association of Official Analytical Chemists, 1975). Crude protein was calculated by multiplying nitrogen by 6.25. Amino acids were analyzed by ion-exchange chromatography following acid hydrolysis. Cystine/cysteine was measured as cystenic acid following performic acid oxidation and acid hydrolysis; tryptophan was measured following alkaline hydrolysis. In vitro dry matter digestibility (IVDMD) was determined by the procedure of Tilley and Terry (1963). Elemental analyses were performed by a dry ash procedure (Jones, 1977) using an inductively coupled plasma spectrograph (Applied Research Laboratories, Inc.). Total and individual cardenolides were extracted into 95% ethanol, cleaned for quantitative analysis by lead acetone precipitation, and assayed by spectroassay and thin-layer chromatography, respectively (Nelson et al., 1981; Brower et al., 1982). Where appropriate, significant differences in means were determined by Duncan's multiple range test (Helwig and Council, 1979).

RESULTS AND DISCUSSION

Cultivation of plant biomass as a source of hydrocarbons or chemical feedstocks has received considerable interest recently (Buchanan et al., 1978a,b; Calvin, 1977, 1979, 1980, 1982). This alternative energy production technology, however, has not attained scale-up development, due in part to a limited data base (Ward, 1982), relatively high energy inputs and production costs (Weisz and Marshall, 1979), and supplies of conventional fuels in developed, industrialized countries. However, in lesser developed arid and semiarid regions of the world, human resources and land are abundant while conventional supplies of energy and food are short (Mann et al., 1977; Hadley and Szarek, 1981). In these areas this technology may provide a viable means of increasing agricultural and industrial production. Cultivation of biomass for liquid fuel supplies and use of processed residues as an animal feed can also potentially reduce desertification in these regions of the world while

Table II. Amino Acid Profiles of Unextracted and Extracted *C. procera*[a]

component	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Tyr	Ala	Arg	Asp	Cys	Glu	Gly	His	Pro	Ser
Unextracted																		
whole plant	3.30[b]	5.78[b]	3.65[b]	1.02[b]	2.99[b]	2.79[b]	1.19[b]	3.97[b]	2.29[b]	3.94[b]	4.59[b]	9.75[b]	1.12[a]	13.86[b]	3.78[b]	1.48[b]	3.70[b]	3.30[c]
stems	2.57[d]	4.27[d]	3.05[c]	0.82[c]	1.78[d]	2.42[c]	0.91[d]	2.95[d]	1.69[d]	3.59[c]	6.12[a]	7.79[d]	1.09[a]	13.11[c]	3.23[c]	1.49[b]	2.69[c]	3.32[b,c]
leaves	3.75[a]	7.20[a]	4.22[a]	1.74[a]	3.67[a]	3.61[a]	1.39[a]	4.33[a]	3.18[a]	5.07[a]	4.48[b,c]	8.01[c]	1.15[a]	10.24[d]	4.79[a]	1.61[a]	4.20[a]	3.74[a]
pods	2.92[c]	4.82[c]	2.84[d]	1.02[b]	2.27[c]	2.26[d]	1.04[c]	3.24[c]	1.94[c]	3.36[d]	4.17[c]	10.32[a]	1.16[a]	16.31[a]	3.38[c]	1.38[c]	2.93[c]	3.45[b]
Hexane Extracted																		
whole plant	3.81[a]	7.10[a]	4.58[a]	1.39[b]	3.65[b]	3.61[a]	1.68[a]	4.46[a]	3.11[b]	5.44[a]	5.23[b]	8.64[b]	1.27[a]	10.26[c]	4.80[a]	1.86[a]	4.03[a]	4.00[a]
stems	2.78[c]	4.60[c]	3.33[c]	0.86[d]	1.95[d]	2.57[b]	0.73[c]	3.19[c]	1.82[d]	3.82[c]	6.38[a]	8.26[c]	1.18[b]	13.72[b]	3.47[c]	1.64[b]	2.71[c]	3.50[b]
leaves	3.77[a]	7.10[a]	4.23[b]	1.78[a]	3.89[a]	3.57[a]	1.66[a]	4.45[a]	3.39[a]	5.08[b]	4.41[c]	7.93[d]	1.10[c]	10.15[c]	4.81[a]	1.61[b]	4.07[a]	3.63[b]
pods	3.23[b]	5.47[b]	3.27[c]	1.04[c]	2.65[c]	2.64[b]	1.28[c]	3.65[b]	2.21[c]	3.80[c]	4.89[b]	10.90[a]	1.31[a]	14.78[a]	3.96[b]	1.64[b]	3.25[b]	3.90[a]
Hexane and Methanol Extracted																		
whole plant	3.54[b]	6.52[b]	4.68[a]	1.42[b]	3.51[b]	3.36[b]	1.49[b]	3.98[b]	2.96[b]	4.50[b]	4.89[c]	8.89[b]	1.34[b]	12.78[a]	4.56[b]	1.79[a]	3.86[a]	3.84[c]
stems	2.95[b]	5.31[d]	4.23[c]	0.89[d]	2.51[d]	3.19[c]	0.88[d]	3.36[c]	2.12[c]	3.81[c]	7.04[a]	9.28[a]	1.50[a]	9.37[d]	4.32[c]	1.71[a]	3.20[b]	4.19[b]
leaves	3.84[a]	7.19[a]	4.36[b]	1.81[a]	3.84[a]	3.66[a]	1.69[a]	4.38[a]	3.27[a]	5.06[a]	4.75[c]	8.35[a]	1.23[c]	10.86[c]	4.96[c]	1.71[b]	3.97[a]	3.78[c]
pods	3.06[c]	4.46[c]	3.27[d]	1.07[c]	2.84[c]	2.57[d]	1.12[c]	3.40[c]	2.25[c]	3.60[d]	5.33[b]	9.38[a]	1.36[b]	11.87[b]	3.99[d]	1.71[b]	3.44[b]	4.19[b]
LSD (0.05)	0.05	0.06	0.06	0.07	0.12	0.10	0.07	0.19	0.12	0.09	0.27	0.14	0.04	0.15	0.12	0.04	0.18	0.10

[a] Amino acid concentrations are expressed in grams per 100 g of protein. Means within each treatment group followed by the same letter are not significantly different ($P < 0.05$). LSD is defined as the least significant difference.

Table III. IVDMD of Unextracted and Extracted *C. procera*

component	extraction solvent[a]	IVDMD[b] mean	SE
whole plant	N	67.4[a]	
	H	71.1[a]	2.0
	H and M	63.3[b]	
stems	N	52.6[a]	
	H	51.7[a]	1.5
	H and M	48.8[b]	
leaves	N	76.5[a]	
	H	80.0[b]	0.5
	H and M	78.7[c]	
pods	N	68.2[a]	
	H	71.2[ab]	1.5
	H and M	71.8[b]	

[a] Soxhlet extracted <9 h; N, H, and H and M are defined as none (unextracted), hexane extract, and hexane followed by methanol extraction, respectively. [b] Means within each component type followed by the same letter are not significantly different (<0.05).

increasing animal protein supplies for human consumption (Dhir, 1977; Kalla et al., 1977; Saxena, 1977; Shankarnarayan, 1977).

C. procera has been shown to be a source of plant hydrocarbons by using a simple, relatively inexpensive solvent extraction technique (Erdman and Erdman, 1981). Solar energy could also be used to power the extraction process, thereby reducing production costs. Farm production of this species would yield a hydrocarbon extract which could be sold for cash by the individual farmer or used as a fuel; the residues could then be recycled through indigenous domestic animal species for the production of animal protein and biological fertilizers.

The proximate analyses of unextracted, hexane-extracted, or hexane and methanol extracted residues are presented in Table I. Similar values for unextracted alfalfa and euphorbia forages are also included for comparative purposes. Generally, significant differences ($P < 0.05$) in residues within and between treatments were observed. Hexane extraction reduced ether extract levels 87–96%, while generally increasing crude protein, ash, and nitrogen-free extract levels. Secondary extraction with methanol generally reduced crude protein, ether extract, ash, and nitrogen-free extract levels, while increasing crude fiber content. The crude protein content of unextracted and extracted whole plants was comparable to that of euphorbia but less than that of alfalfa. Although crude protein digestibility is unknown, except for stems, all residues contained sufficient total crude protein for ewes, gestating ewes, replacement lambs and yearlings, rams weighing less than 60 kg, and finishing lambs (National Academy of Sciences, 1975). Except for stems, all residues contained sufficient total protein for goat maintenance on semiarid rangeland, slightly hilly pastures and during early pregnancy. Similarly, levels were satisfactory for goats on arid rangeland, sparse vegetation, mountainous pastures, and early pregnancy (National Academy of Sciences, 1981). Percent total crude protein content for all residues except stems were satisfactory for all classes of breeding beef cattle, growing and finishing calves, and yearlings greater than 250 kg in weight (National Academy of Sciences, 1976).

The amino acid profiles of unextracted and extracted *C. procera* residues are presented in Table II. Significant differences in amino acid concentrations within and between treatments were observed. Generally, hexane extraction and hexane and methanol extraction resulted in increased amino acid concentrations. Although amino acid availability of *C. procera* is unknown, the whole plant or portions other than stems contain levels of the indispensable amino acids above the percentage requirements for all classes of swine (National Academy of Sciences, 1979b). Amino acid levels were deficient for broilers; however, levels for egg- and meat-type chickens were generally sufficient in the leaf fractions for the acids isoleucine, leucine, lysine, methionine, phenylolanine, threonine, tryptophan, and valine (National Academy of Sciences, 1977a). Except for low methionine and cystine concentrations, amino acid levels in unextracted and extracted leaf residues were satisfactory for growing rabbits. All other plant components, however, were deficient in amino acid concentrations for growing rabbits (National Academy of Sciences, 1977b).

Due in part to the relatively high crude fiber content of residues and the predominance of ruminant domestic animal species in areas indigenous to *C. procera*, the IVDMD was measured to estimate ruminant digestibility. The IVDMD of unextracted and extracted *C. procera* fractions are presented in Table III. The IVDMD was not significantly affected by hexane extraction of whole plants, stems, or pods. Hexane extraction of leaves, however,

Table IV. Mineral Concentration of *C. procera* Fractions[a]

component	μg/g Ca	P	Na	K	Mg	Fe	Cu	Zn	Mn	Al	B
					Unextracted						
whole plant	15883[b]	2310[c]	2643[a]	28163[a]	8188[b]	91[a]	13[b]	31[a]	123[b]	31[a]	51[b]
stems	5451[c]	2010[d]	1811[c]	17753[d]	6096[c]	54[b]	18[a]	15[d]	39[c]	13[b]	19[d]
leaves	20944[a]	2949[b]	2063[b]	24965[c]	11052[a]	103[a]	9[c]	28[b]	264[a]	40[a]	73[a]
pods	2338[d]	3364[a]	1546[d]	26355[b]	3996[d]	99[a]	8[c]	23[c]	33[c]	9[b]	31[c]
					Hexane Extracted						
whole plant	15968[b]	1790[c]	2438[a]	25299[c]	7038[b]	65[c]	12[b]	31[a]	91[b]	17[b]	46[b]
stems	5497[c]	1992[c]	1883[c]	18661[d]	6128[c]	52[c]	18[a]	15[c]	39[c]	12[b]	20[d]
leaves	22267[a]	3020[b]	2203[b]	26859[b]	11590[a]	117[b]	12[b]	29[a,b]	271[a]	50[a]	76[a]
pods	2902[d]	36661[a]	1498[d]	29022[a]	4977[d]	146[a]	9[c]	28[b]	41[c]	13[b]	38[c]
					Hexane and Metanol Extracted						
whole plant	14944[b]	2545[c]	1547[a]	16189[c]	8677[b]	86[b]	10[a,b]	26[c]	150[b]	35[a,b]	55[b]
stems	7328[c]	2543[c]	1107[b]	13679[d]	7466[c]	62[c]	13[a]	18[d]	55[c]	27[b]	25[d]
leaves	25046[a]	3294[b]	1479[a]	21236[b]	12192[a]	120[a]	8[b]	31[b]	295[a]	42[a]	80[a]
pods	3675[d]	4240[a]	1187[b]	27415[a]	5772[d]	89[b]	8[b]	34[a]	59[c]	14[c]	35[c]
LSD (0.05)	510	159	57	732	346	11	2	2	6	9	1

[a] Means within each treatment group followed by the same letter are not significantly different (<0.05). LSD is defined as the least significant difference between similar components for different treatments.

Table V. Concentration of Cardenolide in Unextracted and Extracted *C. procera*[a]

component	total digitoxin equiv, mg/g	μg/g								
		Ascleposide	Calactin	Calotoxin	Calotropin	Calotropagenin	Coroglaucigenin	Proceroside	Uscharidin	Uzarigenin
Unextracted										
whole plant	4.000[a]	190	379	190	569	190	379	190	759	759
stems	3.934[a]	184	184	184	734	184	184	367	1469	184
leaves	1.654[b]	–	183	92	275	183	732	92	–	366
pods	3.255[a]	–	313	–	313	157	1879	–	157	313
Hexane Extracted										
whole plant	3.745[a]	150	225	225	749	225	375	150	899	524
stems	3.783[a]	565	188	188	565	–	188	377	1130	188
leaves	1.383[c]	–	65	195	325	–	195	–	130	391
pods	1.779[b]	–	176	176	263	176	702	–	–	263
Hexane and Methanol Extracted										
whole plant	0.002[a]	–	–	–	+	+	–	–	+	–
stems	0.001[a]	–	–	–	+	+	–	–	+	–
leaves	0.002[a]	–	–	–	+	–	–	–	–	–
pods	0.001[a]	–	–	–	–	+	–	–	–	+
LSD (0.05)	0.682									

[a] Means within each treatment group followed by the same letter are not significantly different ($P < 0.05$). LSD is defined as the least significant difference. A (+) and (–) are defined as trace amount detected and not detected, respectively.

significantly increased IVDMD. Hexane and methanol extraction significantly reduced IVDMD in whole plants and stems while increasing leaves and pods IVDMD.

The concentration of selected minerals is presented in Table IV. Mineral concentration varied with plant component and extraction solvent. Significant differences within and between treatments were observed. Although mineral availability is unknown, total concentrations indicate that all residues contained sufficient potassium, magnesium, copper, and manganese to meet the percentage dietary requirements of bred gilts, sows, and boars (National Academy of Sciences, 1979b). All residues were deficient in phosphorus, while stems and pods were also deficient in calcium (National Academy of Sciences, 1979b). Reported mineral levels were satisfactory for dry and pregnant cows (National Academy of Sciences, 1976).

Total and individual cardenolide concentrations were measured to evaluate the extraction techniques and to determine levels in the potential feed products. The cardenolide concentrations in unextracted and extracted residues are presented in Table V. Comparison of component types within unextracted or hexane and methanol extracted treatments did not differ significantly in total digitoxin equivalent concentrations. However, digitoxin equivalent concentrations in leaves and pods were significantly lower in the hexane-extracted treatment. Hexane-extracted pods were significantly lower in total digitoxin concentration when compared with unextracted pods; however, whole plant, stems, and leaves were not significantly different. Individual cardenolides varied in components within treatments. Only traces of cardenolides were detected in the methanol-extracted residues. Methanol extraction essentially removed all plant cardenolides from the residues which would be expected to decrease the toxic effects of these compounds ingested in feeds.

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Registry No. Ca, 7440-70-2; P, 7723-14-0; Na, 7440-23-5; K, 7440-09-7; Mg, 7439-95-4; Fe, 7439-89-6; Cu, 7440-50-8; Zn, 7440-66-6; Mn, 7439-96-5; Al, 7429-90-5; B, 7440-42-8; ascleposide, 3080-19-1; calactin, 20304-47-6; calotoxin, 20304-49-8; calotropin, 1986-70-5; calotropagenin, 24211-64-1; coroglaucigenin, 468-19-9; proceroside, 25323-74-4; uscharidin, 24321-47-9; uzarigenin, 466-09-1; digitoxin, 71-63-6.

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Plakalbumin Converts to Heat-Stable Form under the Same Condition as an Ovalbumin–s-Ovalbumin Transformation

Shinji Shitamori and Ryo Nakamura*

A solution of plakalbumin, a protein derived from ovalbumin by mild hydrolysis with subtilisin, was adjusted to pH 9.9 and kept at 55 °C for 16 h. The denaturation temperature of plakalbumin measured by differential scanning calorimetry before and after this treatment was 77.2 and 86.6 °C, respectively. The circular dichroism of both kinds of plakalbumin was almost the same, the isoelectric focusing of plakalbumin became broad, and the surface hydrophobicity of plakalbumin increased slightly with the above treatment. All these results suggest that plakalbumin converts to the heat-stable form (s-plakalbumin) after the manner of an ovalbumin–s-ovalbumin transformation.

Ovalbumin has been shown to change to a heat-stable form (s-ovalbumin) during the storage of shell eggs (Smith, 1964). This conversion also occurs in an isolated ovalbumin solution, the rate increasing with pH and temperature (Smith and Back, 1965). Although slight conformational changes were detected by Raman difference spectroscopy (Kint and Tomimatsu, 1979), the gross conformation of ovalbumin and s-ovalbumin was almost the same (Smith and Back, 1968; Nakamura et al., 1980).

In previous studies (Nakamura et al., 1980, 1981; Nakamura and Ishimaru, 1981), the authors compared the properties of ovalbumin with those of s-ovalbumin and found some differences; the isoelectric focusing of s-ovalbumin became broader than that of ovalbumin whereas their polyacrylamide gel electrophoretic pattern was identical. Also, the intrinsic viscosity of s-ovalbumin was lower than that of ovalbumin, whereas its hydrophobicity was greater. The relationship of these differences between both proteins with the increased thermal stability of s-ovalbumin, however, is not clear.

It is of interest to know whether proteins other than ovalbumin can be converted to the heat-stable form under the same condition as for ovalbumin–s-ovalbumin transformation. Our previous study on several proteins revealed no conversion to the heat-stable form (Nakamura et al., 1980). The present investigation provides results on the conversion of plakalbumin, a protein derived from ovalbumin by mild proteolysis with subtilisin (Linderstrøm-Lang and Ottesen, 1947), to the heat-stable form by heating at 55 °C for 16 h under the pH of 9.9. This form

Faculty of Agriculture, Nagoya University, Chikusa-ku, Nagoya 464, Japan.

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